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Calcemic response to parathyroid hormone in renal failure: Role of phosphorus and its effect on calcitriol

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Calcemic response to parathyroid hormone in renal failure: Role of phosphorus and its effect on calcitriol. The calcemic response to parathyroid hormone (PTH) is decreased in renal failure. The reduction of hyperphosphatemia improves the calcemic response to PTH in animals with advanced renal failure. However, since low calcitriol levels in renal failure may also contribute to the decreased calcemic response to PTH, the improved calcemic response observed during the reduction of serum phosphorus may be partially mediated by an increase in serum calcitriol levels. The present study evaluated the calcemic response to PTH in rats with moderate and advanced renal failure and how this response was modified by a high and a low phosphorus diet. In addition, the effect of a change in dietary phosphorus on calcitriol levels was also evaluated. A 48-hour continuous infusion of 1-34 rat PTH increased the serum calcium level to 18.2 ± 0.4 mg/dl in normal rats, versus 13.7 ± 0.9 and 12.1 ± 0.2 mg/dl in rats with moderate and advanced renal failure, respectively. During the PTH infusion, a high phosphorus diet increased the serum phosphorus and resulted in a reduced calcemic response to PTH at each level of renal function; respective serum calcium levels were 13.8 ± 0.6 mg/dl in normals, 11.2 ± 0.2 mg/dl in moderate renal failure and 9.6 ± 0.5 mg/dl in advanced renal failure. In normal rats and in rats with moderate renal failure, dietary phosphorus restriction during the PTH infusion increased serum calcitriol levels. In rats with advanced renal failure, serum calcitriol levels were lower than in the other two groups and were not affected by changes in dietary phosphorus. In an additional group of rats, parathyroidectomy corrected the calcemic response to PTH, suggesting that resistance to the calcemic action of PTH in renal failure requires the presence of circulating levels of PTH. In conclusion, a decreased calcemic response to PTH was present not only in advanced renal failure but also in moderate renal failure. This abnormal response to PTH appears to be multifactorial. Phosphorus restriction improved the calcemic response to PTH and this effect could have been in part due to higher values of calcitriol; however, in advanced renal failure, calcitriol levels were not influenced by dietary phosphorus, and thus, the increased calcemic response to PTH induced by phosphorus restriction was independent of calcitriol. Finally, down regulation of bone receptors for PTH appears to play a major role in the decreased calcemic response to PTH in renal failure.

Resistance to the calcemic action of parathyroid hormone (PTH) has been implicated as a factor in the development of both hypocalcemia and secondary hyperparathyroidism in chronic renal failure [1]. Resistance to the calcemic action of PTH in renal failure was first reported by Evanson in 1966 [2], and subsequently confirmed by others in both acute and chronic renal failure [3–5]. However, the mechanism(s) responsible for the resistance to the calcemic action of PTH have been disputed. Postulated mechanisms include: 1) phosphate retention [6]; 2) decreased serum levels of vitamin D metabolites [7]; and 3) down regulation of bone cell receptors for PTH due to high serum PTH levels [8].

Studies in uremic rats by Somerville and Kaye have shown that an increase in serum phosphorus produced skeletal resistance to the calcemic action of PTH [6]. However, in dogs with chronic renal failure, Kaplan et al observed that phosphate restriction, sufficient to prevent phosphate retention, did not correct the resistance to the calcemic action of PTH [9].

Considerable evidence has accumulated both for and against vitamin D metabolites as a factor in the skeletal resistance to the calcemic action of PTH. Massry et al observed that calcitriol administration partially corrected the resistance to the calcemic action of PTH in rats with either acute or chronic renal failure [5]. In another study, these investigators also observed that the administration of calcitriol together with $24,25(OH)_2D_3$ corrected the calcemic response to PTH [7]. However, other investigators have not been able to confirm that calcitriol improves the calcemic response to PTH [8].

In renal failure, the relative effects of hyperphosphatemia and low calcitriol levels on the resistance to the calcemic action of PTH are still unclear. Since the production of calcitriol is modulated by the serum phosphorus, the effect of phosphorus on the calcemic response to PTH may be mediated by changes in calcitriol levels.

In most of the previous studies in which the calcemic response to PTH was evaluated, the infusion of PTH was for a relatively short period of time (5 to 12 hours) and only one factor was evaluated. In addition, only animals with advanced renal failure were studied; thus, little information is available about the calcemic response to PTH in moderate renal failure and during a longer PTH infusion.

Another factor to be considered in the decreased calcemic response to PTH is the effect of "down regulation" which occurs as a consequence of the increase in PTH levels. In a previous study, thyroparathyroidectomy normalized the calcemic response to PTH in renal failure [8].

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The goal of the present study was to evaluate: 1) if resistance to the calcemic action of PTH was present, not only in advanced, but also in moderate renal failure; 2) the effect of changes in dietary phosphorus on the calcemic response to PTH; 3) whether phosphorus induced changes in the calcemic response to PTH could be mediated by changes in serum levels of calcitriol; and 4) the effect of high levels of PTH on the calcemic response to PTH in advanced renal failure.

Our findings suggest that both phosphorus and the degree of renal failure independently affect the calcemic response to PTH. Changes in calcitriol may partially mediate the effect of phosphorus on the calcemic response to PTH in normal and moderate renal failure rats. However, in advanced renal failure, serum calcitriol levels were not influenced by changes in serum phosphorus. Finally, the removal of circulating PTH by selective parathyroidectomy in rats with advanced renal failure corrected the calcemic response to PTH.

Methods

Male Wistar rats weighing 125 to 140 grams were divided into three groups: the first group underwent sham operation, the second group ligation of one main artery in the hilum of the left kidney, followed one week later by right nephrectomy; and the third group, ligation of two main arteries in the hilum of the left kidney, followed one week later, by right nephrectomy. The intent was to produce different degrees of renal failure in the second and third groups.

All rats were placed on a diet containing 0.6% calcium and 1.2% phosphorus with 100 IU/100 g of vitamin D. This high phosphorus diet has been shown to exacerbate secondary hyperparathyroidism in rats with renal failure [10, 11]. The rats were maintained in individual cages and pair-fed. The daily food allowance was adjusted to the intake of the lowest group; however, rats consistently eating less than 12 grams per day were removed from the study. Seventeen days after the initiation of the high phosphorus diet, blood was obtained for measurement of creatinine, phosphorus, calcium, and PTH. Based on the serum creatinine concentration, rats were assigned to the following groups:

Normal renal function—serum creatinine less than 0.5 mg/dl;

Moderate renal failure—serum creatinine 0.5 to 0.6 mg/dl; and

Advanced renal failure—serum creatinine greater than 0.6 mg/dl.

Twenty days after the right nephrectomy, rats were fasted for 12 hours; then rat PTH 1-34 (Bachem, Torrance, California, USA) from a single lot was infused at a constant rate of 1.3 U/100 g/hr for 48 hours via a subcutaneously implanted miniosmotic pump (Alza, Palo Alto, California, USA; model 2001). The pumps were filled with PTH in isosmotic saline with 2% cysteine and HCl added to achieve a pH 1.5. Blood for calcium, phosphorus and creatinine was obtained immediately before the placement of the miniosmotic pump, and 25 and 48 hours after the pump placement. Since measurement of calcitriol requires a large aliquot of serum, a separate group of rats were sacrificed before the PTH infusion and the blood used for calcitriol measurement. Calcitriol was also measured at sacrifice after the 48 hours of PTH administration.

Since the model 2001 miniosmotic pump maintains a constant delivery of one microliter per hour and the filling volume is 200 microliters, its functional life-span is at least seven days. Thus, after 48 hours of infusion, each pump was removed and implanted in a rat from a different group; as a result, the same pump was used in a different group of rats in a random order. Each pump was used in three rats. During the PTH infusion, rats were housed in individual metabolic cages and urine was collected for measurement of volume, calcium, phosphorus and creatinine.

Effect of renal failure

To evaluate the effect of renal failure on the calcemic response to PTH, rats with normal renal function ($N = 10$), rats with moderate renal failure ($N = 9$) and rats with advanced renal failure ($N = 13$) received a PTH infusion as previously described. To separate the effect of the magnitude of renal failure from that of phosphorus retention, all rats received a low phosphorus (0.2%) diet (LPD) during the PTH infusion. To minimize intestinal absorption of calcium, the diet was calcium free.

Effect of phosphorus retention

To evaluate the role of serum phosphorus on the calcemic response to PTH, rats from each level of renal function underwent a PTH infusion while receiving either an LPD or a high phosphorus diet (1.0% P, 0% Ca) (HPD). To evaluate the effect that changing dietary phosphorus independent of a PTH infusion, may have on the serum calcium concentration, rats with advanced renal failure were studied during the ingestion of either a LPD ($N = 7$) or HPD ($N = 7$) for a 48 hours period; PTH was not infused in these rats.

Changes in serum calcitriol

These were assessed by determination of serum calcitriol in normal rats, and in rats with moderate and advanced renal failure before and after 48 hours of PTH infusion. Serum calcitriol was studied during both a LPD and HPD.

Effect of parathyroidectomy

To determine the role of high PTH levels on the decreased calcemic response to PTH, rats with advanced renal failure underwent selective parathyroidectomy (PTX) ($N = 7$) two days before the infusion of PTH. To prevent a fall in serum calcium during the two days following PTX, rats were placed on a high calcium diet (Ca:1.3%, P:0.8%). During the PTH infusion, these rats ingested a LPD.

Biochemical determinations

Serum calcium was measured by atomic absorption (Perkin Elmer, Norwalk, Connecticut, USA), serum phosphorus with a specific kit (Sigma, St Louis, Missouri, USA); serum creatinine with a creatinine analyzer (Beckman Instruments, Fullerton, California, USA) and serum PTH with an N-terminal radioimmunoassay (Nichols Institute, San Juan Capistrano, California, USA). This assay has been previously validated for the determination of circulating PTH in the rat [11–13]. Serum levels of calcitriol were measured using a radioreceptor assay (Nichols Institute); the intra-assay and inter-assay coefficient of variation were 6.8% and 8.5%, respectively. A comparison of this assay with conventional radioreceptor assay after double column

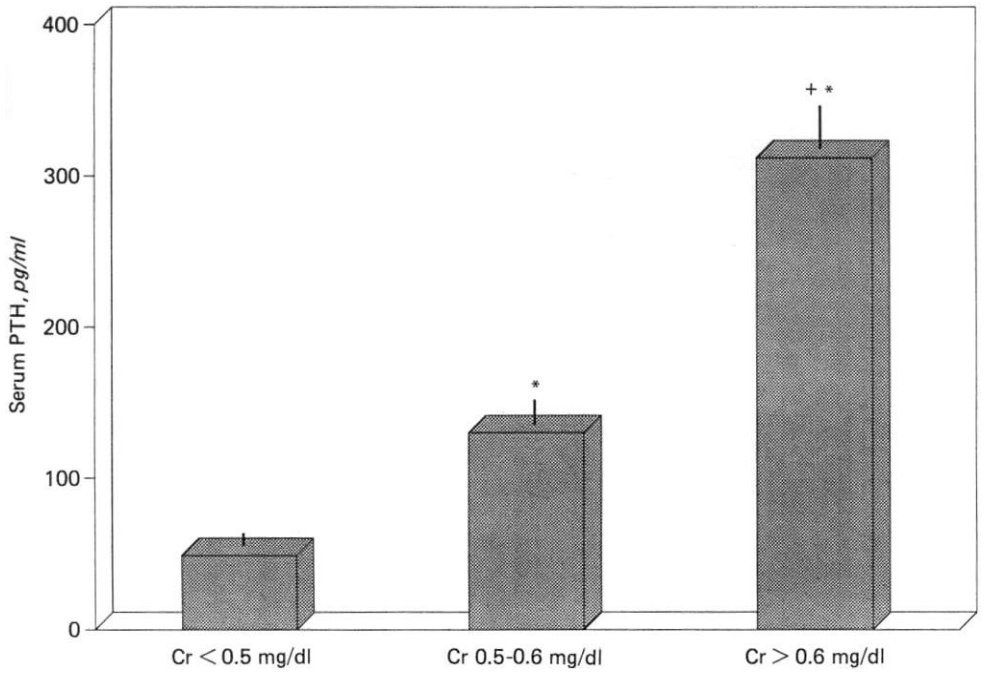

Fig. 1. *Serum levels of PTH in the three groups of rats: normals (Cr < 0.5 mg/dl), moderate (Cr 0.5–0.6 mg/dl) and advanced renal failure (Cr > 0.6 mg/dl).* Values are mean ± SE. (*) $P < 0.01$ vs. Cr < 0.5 mg/dl, (+) $P < 0.01$ vs. Cr 0.5–0.6 mg/dl.

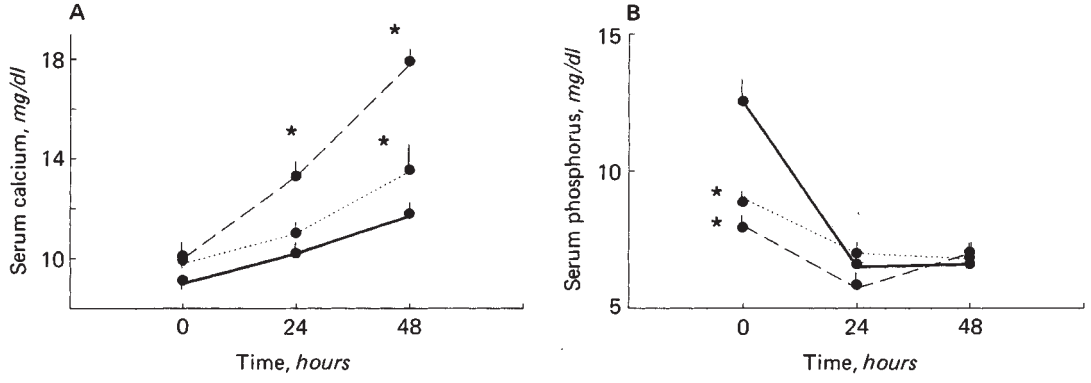

Fig. 2. *Changes in serum calcium (A) and phosphorus (B) during a 48-hour continuous PTH infusion in the three groups of rats.* Values are mean ± SE. (*) $P < 0.05$ vs. Cr < 0.5 mg/dl. During the PTH infusion, rats were ingesting a low phosphorus diet.

separation showed a significant correlation approaching identity ($r = 0.93$, $N = 32$).

Statistics

Comparisons of the means was made by ANOVA followed by the Duncan test. The unpaired *t*-test was used to compare two means of different groups. Results are expressed as mean ± standard error (SE).

Results

Effect of renal failure

Rats with moderate and advanced renal failure had creatinine clearances of 5.1 ± 0.5 ml/min/kg and 2.5 ± 0.2 ml/min/kg, respectively; these were significantly lower ($P < 0.01$) than 10.1 ± 0.9 ml/min/kg in normal rats. The basal serum PTH levels in the three groups of rats are shown in Figure 1. PTH levels were highest in rats with advanced renal failure; in addition, the PTH level was higher in the group with moderate renal failure than normals.

The effect of a 48-hour PTH infusion on serum calcium and phosphorus is shown in Figure 2. During the PTH infusion, rats were maintained on a LPD. In normal rats, the PTH infusion increased the serum calcium to 18.2 ± 0.4 mg/dl; this value was significantly greater than in rats with moderate (13.7 ± 0.9 mg/dl) and advanced (12.1 ± 0.2 mg/dl) renal failure. At baseline, the rats with advanced renal failure had a significantly lower serum calcium concentration (8.9 ± 0.2 mg/dl, $P < 0.01$) and a significantly greater serum phosphorus concentration (12.1 ± 0.8 mg/dl, $P < 0.01$) than either normal rats or rats with moderate renal failure. The infusion of PTH during a LPD produced a decrease in serum phosphorus. At 24 hours, the serum phosphorus was similar in the three groups and this was maintained until the end of the PTH infusion (Fig. 2). As shown in Table 1, differences in PTH-induced hypercalcemia could not

Table 1. Daily urinary excretion of calcium (mg), in rats with different levels of serum creatinine (S_{Cr}) during a 48 hour infusion of PTH and receiving a low phosphorus diet (LPD; P = 0.2%, Ca = 0%) or a high phosphorus diet (HPD; P = 1.0%, Ca = 0%)

	LPD			HPD		
S_{Cr} *mg/dl*	<0.5	0.5–0.6	>0.5	<0.5	0.5–0.6	>0.6
24 hr	1.1 ± 0.3[a]	1.4 ± 0.4	0.8 ± 0.1[a]	0.1 ± 0.03	0.4 ± 0.1	0.3 ± 0.06
48 hr	7.5 ± 2.2[a]	2.9 ± 0.4[a]	2.0 ± 0.4[a]	1.6 ± 0.6	0.9 ± 0.2	0.8 ± 0.20
N	10	9	13	12	10	13

N, number of rats in each group.
[a] $P < 0.05$ as compared with HPD

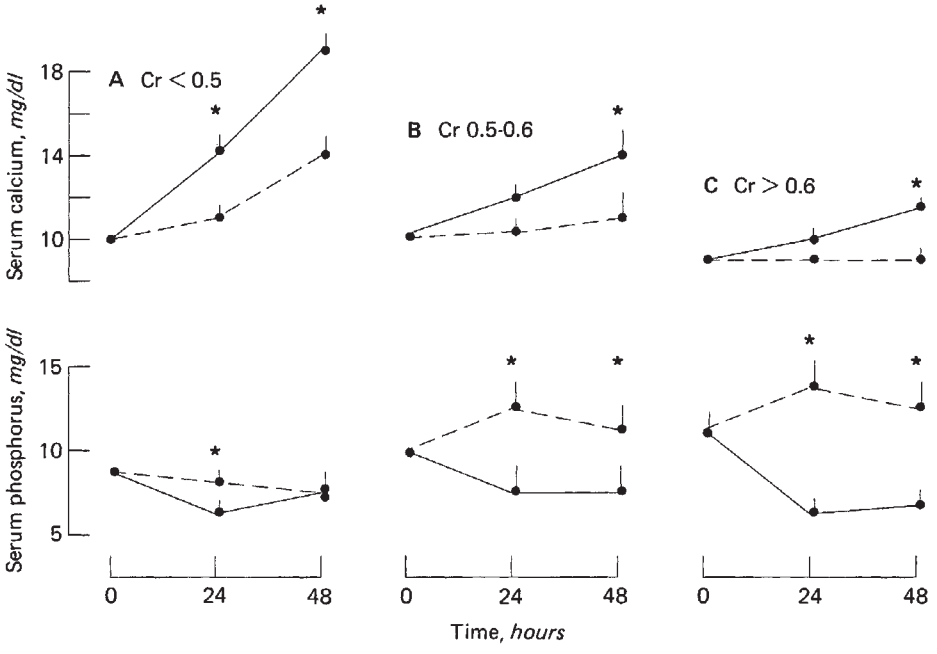

Fig. 3. *Changes in serum calcium and phosphorus during a 48-hour continuous PTH infusion in the three groups of rats studied:* (**A**) *normals (Cr < 0.5 mg/dl),* (**B**) *moderate renal failure (Cr = 0.5–0.6 mg/dl), and* (**C**) *advanced renal failure (Cr > 0.6 mg/dl).* Continuous line (—) indicates ingestion of a low phosphorus diet and the dotted line (---) ingestion of a high phosphorus diet during the PTH infusion. Values are mean ± SE, (*) $P < 0.05$ vs. high phosphorus diet.

be attributed to changes in urinary calcium excretion. Normal rats, which had the greatest hypercalcemic response to PTH, excreted more calcium in a 24 hour urine than rats with moderate or advanced renal failure.

Effect of phosphorus

Figure 3 displays the effect of a low and a high phosphorus diet on the calcemic response to a PTH infusion. At each level of renal function, the HPD decreased the calcemic response to PTH.

In rats with normal renal function the serum phosphorus was lower at 24 hours in rats on a LPD than on a HPD (5.4 ± 0.5 vs. 7.7 ± 0.3 mg/dl, $P < 0.01$). At 48 hours, the serum phosphorus levels were similar on a LPD or HPD (7.2 ± 0.9 and 7.5 ± 0.4 mg/dl, respectively). Despite similar serum phosphorus concentration at 48 hours, the calcemic response to PTH was decreased in rats ingesting the HPD. The respective serum calcium levels in normal rats were 13.8 ± 0.6 vs. 18.2 ± 0.4 mg/dl ($P < 0.001$) at 48 hours.

In rats with moderate renal failure on a HPD the serum phosphorus concentration was greater than in rats on a LPD (Fig. 3). Similar to normal rats, the HPD decreased the calcemic response to PTH. On a HPD, the serum calcium increased to 10.4 ± 0.2 mg/dl at 24 hours; this was marginally different than that of rats on a LPD, 11.1 ± 0.3 mg/dl ($P = 0.07$); however, by 48 hours, the serum calcium level was significantly lower on a HPD, 11.2 ± 0.2 mg/dl, than on a LPD, 13.7 ± 0.9 mg/dl ($P < 0.01$).

In rats with advanced renal failure, the serum phosphorus level in the rats ingesting a HPD was 13.9 ± 1.7 and 12.9 ± 1.4 mg/dl at 24 and 48 hours, respectively; on a LPD, serum phosphorus levels were significantly lower (6.1 ± 0.3 and 6.8 ± 0.4 mg/dl) at 24 and 48 hours, respectively ($P < 0.01$). As compared with the LPD, the calcemic response to PTH at 48 hours was decreased on an HPD, 12.0 ± 0.2 versus 9.6 ± 0.5 mg/dl ($P < 0.001$).

In rats with advanced renal failure, the change in serum calcium, phosphorus, and calcitriol levels induced by a HPD and a LPD during the presence or absence of a PTH infusion is shown in Figure 4. During a LPD, a PTH infusion increased the magnitude of the increment in serum calcium from 1.44 ± 0.60 to 3.26 ± 0.41 mg/dl, $P < 0.01$; this was associated with a decrease ($P < 0.01$) in serum phosphorus that was similar in both groups. On a HPD, the PTH infusion did not produce significant changes in serum calcium or phosphorus. Serum calcitriol levels were unaffected by changes in dietary phosphorus and the infusion of PTH.

For all three groups, the total amount of calcium excreted in

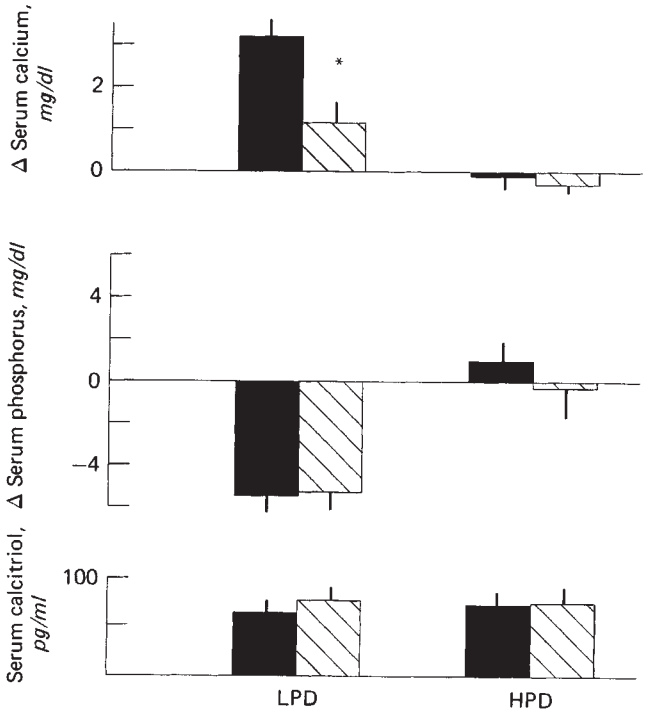

Fig. 4. *Changes in serum calcium and phosphorus, and concentration of serum calcitriol in rats with advanced renal failure after 48 hours ingesting a low (LPD) or a high (HPD) phosphorus diet, with (■) or without (▨) administration of PTH.* $N = 7$ in each of the two LPD groups and 13 in each of the HPD groups. Values are mean ± SE and (*) $P < 0.01$.

the urine during the PTH infusion was significantly greater in rats ingesting a LPD than in those on a HPD (Table 1). This reflected the differences in the serum calcium concentrations. The amount of phosphorus excreted in the urine was increased in rats ingesting a HPD (Table 2). For a given diet, urinary excretion of phosphorus decreased with the severity of renal failure.

Changes in serum calcitriol

Serum levels of calcitriol before and after the PTH infusion are shown in Figure 5; renal failure resulted in a progressive decrease in calcitriol levels ($P < 0.01$). In normal rats, the PTH infusion increased calcitriol levels and this response was augmented by the low phosphorus diet despite no significant decrease in serum phosphorus at 48 hours. In rats with moderate renal failure, the PTH infusion did not increase calcitriol levels during the HPD, but there was a significant increase on the LPD. In this group, the differences in dietary phosphorus resulted in significant differences in serum levels of calcitriol (121 ± 25 pg/ml on HPD vs. 198 ± 22 pg/ml on LPD, $P < 0.05$). In rats with advanced renal failure, serum levels of calcitriol were lowest and did not increase significantly after the PTH infusion irrespective of the phosphorus content of the diet (72 ± 20 pg/ml on HPD vs. 64 ± 19 pg/ml on LPD). Those values were not different from calcitriol levels obtained in rats with advanced renal failure on a HPD or a LPD for 48 hours without a PTH infusion, 78 ± 35 and 82 ± 29 pg/ml, respectively (Fig. 4).

Effect of parathyroidectomy

In PTX rats with advanced renal failure on an LPD, PTH infusion increased the serum calcium from 8.10 ± 0.61 to 16.0 ± 0.89 mg/dl, $P < 0.001$. The mean increase in serum calcium in these rats (7.88 ± 0.50 mg/dl) was similar to the increase (8.69 ± 0.34 mg/dl) in normal rats after a PTH infusion and on a LPD (Fig. 6). By contrast, in rats with advanced renal failure and secondary hyperparathyroidism, the PTH infusion produced an increase in serum calcium of only 3.10 ± 0.24 mg/dl; this was significantly less ($P < 0.01$) than PTX rats with the same degree of renal failure.

After the PTH infusion, the serum phosphorus level in PTX rats with advanced renal failure was 7.3 ± 0.7 mg/dl. This concentration was similar to the post-PTH infusion in normal rats, 7.2 ± 0.9 mg/dl, and in parathyroid-intact rats with advanced renal failure (6.5 ± 0.3 mg/dl).

Discussion

The results of this study show that the calcemic response to PTH was decreased in renal failure. The degree of renal failure and the level of serum phosphorus were factors that independently modified the calcemic response to PTH. Moreover, this study demonstrates that a decreased calcemic response to a 48 hour infusion of PTH was present at moderate renal failure. In normal rats, a change in dietary phosphorus was an important factor in the calcemic response to PTH, even without concomitant changes in serum phosphorus. In normal rats and in rats with moderate renal failure, dietary phosphorus restriction increased serum calcitriol levels which may have contributed to the improved calcemic response to PTH. However, in advanced renal failure, the effect of phosphorus was independent of calcitriol. In these rats, the removal of circulating PTH by parathyroidectomy corrected the calcemic response to PTH.

Effect of renal failure

Renal failure resulted in a progressive increase in PTH and a decrease in the calcemic response to PTH. These findings are in agreement with previous observations that hyperparathyroidism is present even in early renal failure [14–16]. As compared with normal rats, rats with moderate and advanced renal failure had a decreased calcemic response to PTH. Despite a LPD and similar serum phosphorus levels, rats with moderate and advanced renal failure had less urinary excretion of phosphorus than normal rats. Thus, it is likely that phosphorus retention was in part responsible for the decreased calcemic response to PTH in rats with renal failure.

Effect of parathyroidectomy

An important factor responsible for the impaired calcemic response to PTH appears to be down regulation of bone cell receptors due to high PTH levels [8]. Thus, the highest PTH levels observed in rats with advanced renal failure may have contributed to the greatest impairment in the calcemic response to PTH. This is suggested by the fact that removal of circulating PTH by PTX corrected the calcemic response. In a previous study we have shown that after parathyroidectomy, the calcemic response to PTH was similar at any level of renal function [17]. The correction of the calcemic response to PTH after PTX

Table 2. Daily urinary excretion of phosphorus (mg), in rats with different levels of serum creatinine (S_{Cr}) during a 48 hour infusion of PTH and receiving a low phosphorus diet (LPD; P = 0.2%, Ca = 0%); or a high phosphorus diet (HPD; P = 1.0%, Ca = 0%)

	LPD			HPD		
S_{Cr} *mg/dl*	<0.5	0.5–0.6	>0.6	<0.5	0.5–0.6	>0.6
24 hr	35 ± 4	32 ± 4	31 ± 12	104 ± 8	122 ± 12	81 ± 8[a,b]
48 hr	32 ± 4	19 ± 2[a]	16 ± 2[a]	173 ± 10	136 ± 8[a]	106 ± 6[a,b]
N	10	9	13	12	10	13

N, number of rats in each group.
[a] $P < 0.05$ as compared with $S_{Cr} < 0.5$ on the same diet
[b] $P < 0.05$ as compared with S_{Cr} 0.5–0.6 on the same diet

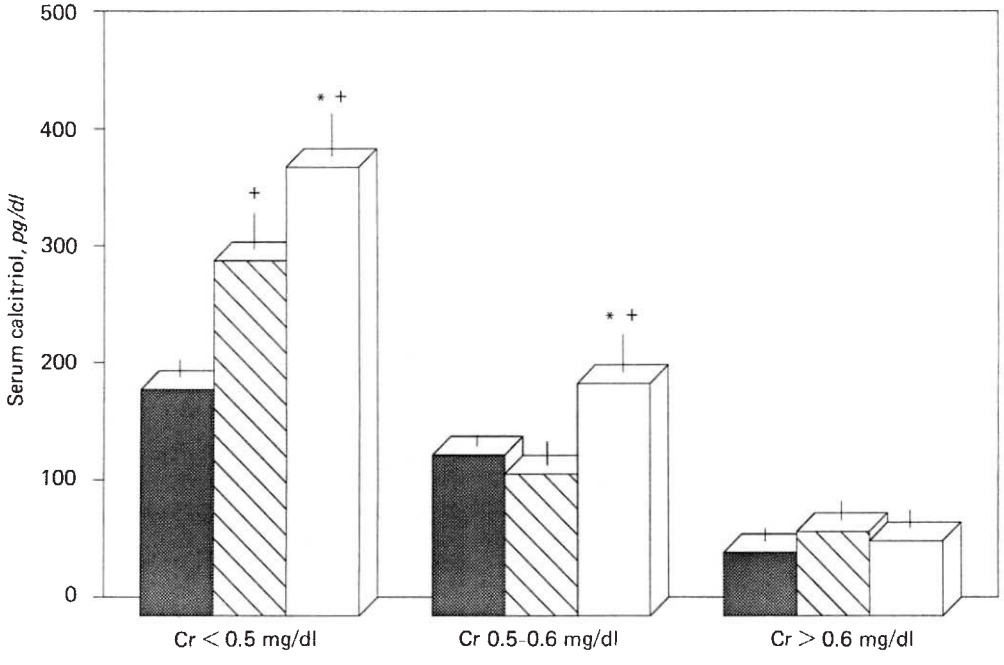

Fig. 5. *Serum calcitriol levels in the three groups of rats before (Pre-PTH, ▩) and after (Post-PTH) a 48-hour PTH infusion during the ingestion of either a high (HPD, ▨) or a low phosphorus diet (LPD, □).* Values are mean ± SE, (+) $P < 0.05$ vs. Pre-PTH, (*) $P < 0.05$ vs. Post-PTH-HPD. The number of rats (*N*) in each group is as follows: Cr < 0.5 pre-PTH, $N = 6$; Cr < 0.5 post-PTH-HPD, $N = 5$; Cr < 0.5 post-PTH-LPD, $N = 7$; Cr 0.5–0.6 pre-PTH, $N = 5$; Cr 0.5–0.6 post-PTH-HPD, $N = 5$; Cr 0.5–0.6 post-PTH-LPD, $N = 6$; Cr > 0.6 pre-PTH, $N = 8$; Cr > 0.6 post-PTH-HPD, $N = 7$; Cr > 0.6 post-PTH-LPD, $N = 7$.

substantiates that there is a down regulation of PTH receptors in bone cells.

Effect of phosphorus

Differences in dietary phosphorus produced significant changes in the calcemic response to PTH. A decrease in dietary phosphorus was associated with an increase in the calcemic response to PTH at all three levels of renal function. In normal rats and in rats with moderate renal failure, it is difficult to separate a direct effect of phosphorus from an indirect effect on calcitriol synthesis. In these rats, increases in the dietary phosphorus intake resulted in decreased calcitriol levels. Thus, an indirect role for phosphorus through a decrease in calcitriol levels must be considered, especially in normal rats, since the serum phosphorus level was not changed at the end of the PTH infusion. However, in rats with advanced renal failure, dietary induced changes in serum phosphorus did not modify calcitriol levels. Thus, in this group, it would appear that the effect of phosphorus on the calcemic response was independent of changes in serum calcitriol.

The failure of phosphorus restriction to stimulate calcitriol in advanced renal failure has been observed previously in humans by Lucas et al [18] and in dogs by Lopez-Hilker et al [19]. Our results suggest that calcitriol cannot be stimulated in rats with advanced renal failure and secondary hyperparathyroidism since two stimulatory factors, phosphorus restriction and a PTH infusion, did not increase serum calcitriol levels. The lack of stimulation in these rats could in part be due to the fact that calcitriol was maximally stimulated by high PTH in the baseline state before the infusion of exogenous PTH.

Even without the administration of PTH, the serum calcium increased in rats with advanced renal failure in response to a low phosphorus diet. This suggests that for a given PTH concentration, the calcium released from bone is increased as the serum phosphorus is decreased.

Our findings in regard to the effect of phosphorus on the calcemic response to PTH are in agreement with those of Somerville and Kaye [6], who found that the removal of phosphorus restored the calcemic response to PTH. However, our results differ somewhat inasmuch as a decrease in the serum phosphorus concentration only partially reversed the impaired calcemic response to PTH. The differences between our results

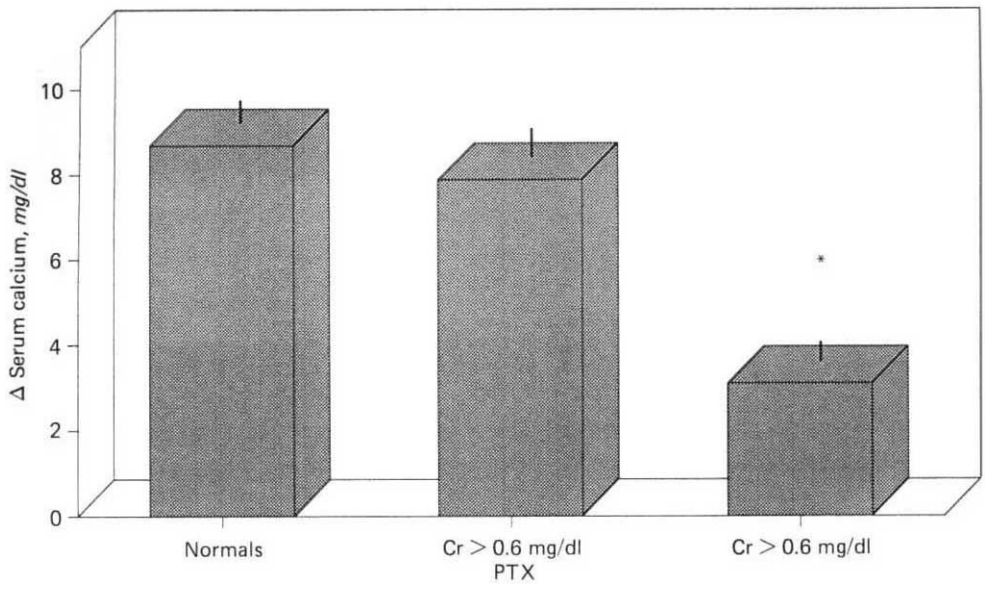

Fig. 6. *Changes in serum levels of calcium in normal rats (Normals), rats with renal failure and parathyroidectomy (Cr > 0.6 mg/dl, PTX) and rats with renal failure without parathyroidectomy (Cr > 0.6 mg/dl).* Values are mean ± SE. (*) $P < 0.01$.

and those of Somerville and Kaye may be due to the fact that they used a rat model of renal failure in which thyroparathyroidectomy was performed. In our study, the decrease in serum or dietary phosphorus improved but did not correct the calcemic response to PTH; however, parathyroidectomy corrected the calcemic response. In the present study, a high phosphorus diet reduced the calcemic response to PTH in rats with normal renal function. This occurred despite a similar serum phosphorus level at the end of the PTH infusion. During the ingestion of a HPD, the PTH induced increment in serum calcium was diminished by almost fifty percent. One possible explanation is that the bone compartment containing readily exchangeable calcium may have been saturated with phosphorus reducing the availability of calcium. In in vitro studies, calcium released from the bone of isolated perfused rats tails was reduced as the phosphorus content of the perfusate was increased; this occurred despite no reduction in PTH stimulated cAMP production [20]. Furthermore, a recent in vitro study shows that the increase in extracellular phosphate directly inhibits bone resorption by osteoclasts [21].

An increase in the calcium-phosphorus product resulting in calcium precipitation cannot be ruled out as a cause of the impaired calcemic response in rats fed a high phosphorus diet. However, the fact that the high phosphorus diet decreased the calcemic response in normal rats despite no significant change in serum phosphorus argues against the importance of calcium precipitation at least in this group. A more likely possibility is the difference in the serum calcitriol levels; both PTH and phosphorus restriction are known to increase calcitriol production [1, 22–27]. As compared to rats on a low phosphorus diet, we observed that a high phosphorus diet reduced the PTH-induced elevation of serum calcitriol levels in normal rats and in rats with moderate renal failure. In some previous studies, calcitriol has improved the calcemic response to PTH [5, 7]; however, this beneficial effect has only been reported in renal failure.

In patients with moderate renal failure, Llach and Massry observed that phosphorus restriction improved the calcemic response to PTH [28]; this was associated with an increased calcitriol level which may have been a factor in the improved calcemic response to PTH. However, phosphorus restriction also resulted in reduced PTH levels. Since high PTH levels may down regulate bone cells receptors for PTH [7], a decrease in PTH levels induced by phosphorus restriction may have contributed to the improved calcemic response to PTH.

In conclusion, in rats with moderate and advanced renal failure there was a decrease in the calcemic response to PTH. This was observed only if circulating PTH was present, suggesting that high PTH levels may impair the calcemic response due to down regulation of PTH receptors in bone cells. High dietary phosphorus increased serum phosphorus levels and at each level of renal function, further decreased the calcemic response to PTH. In normal rats and rats with moderate renal failure, the effect of phosphorus on the calcemic response to PTH may in part be due to changes in serum levels of calcitriol. However, in advanced renal failure, calcitriol levels were not modified by dietary phosphorus and thus, the effect of phosphorus on the calcemic response to PTH may be independent of serum calcitriol levels.

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